QUANTITATION OF BENZO[A] PYRENE DIOLEPOXIDE-AND NNK-INDUCED ADDUCTS AT SPECIFIC GUANINE NUCLEOBASES WITHIN K-RAS AND P53 GENE SEQUENCES

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The mutagenicity of a the prominent tobacco carcinogen benzo[a]pyrene (B[a]P) is believed to result from the chemical reactions of its diepoxide metabolite, (+)-*anti*-7*r*,8*t*-dihydroxy-*c*9,10-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene (BPDE), with DNA, to produce promutagenic lesions, e.g. (+)-*trans-anti* 7*R*,8*S*,9*S*-trihydroxy-10*S*-(N^2-deoxyguanosyl)-7,8,9,10-tetrahydro benzo[a]pyrene (N^2-BPDE-dG). Previous studies using UvrABC endonuclease and ligation mediated PCR (LMPCR) suggested an increased reactivity of BPDE towards guanine nucleobases within codons 157, 158, 248, and 273 of the *p53* tumor suppressor gene. These sites are also the "hot spots" for mutations observed in lung tumors of smokers, suggesting an involvement of B[a]P in the initiation of smoking-induced lung cancer. In the present work, we performed a direct quantitation of N^2-BPDE-dG and NNK adduct *N*7-methylguanine formed at specific guanine nucleobases within *p53* and *K-ras* derived DNA sequences using stable isotope labeling HPLC-ESI-MS/MS. Within *K-ras* derived DNA sequences, increased formation of BPDE-dG was observed at the first guanine of codon 12 (GGT), in agreement with the large number of G → T transversions observed at this position. Interestingly, the amounts of *N*7-methylguanine lesions were the highest at the second position of *K-ras* codon 12, the major "hotspot" for G → A transitions. BPDE reactivity towards CpG sites within *p53* gene was stimulated by the presence of 5-Me group at the neighboring cytosines. The results of this work will help link the initiating mutations observed in lung tumors to specific carcinogens present in tobacco smoke, leading to the development of mechanism-based biomarkers of lung cancer risk in smokers.

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